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Characterization of a Candidate *bcl-1* Gene

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The t(11;14)(q13;q32) translocation has been associated with human B-lymphocytic malignancy. Several examples of this translocation have been cloned, documenting that this abnormality joins the immunoglobulin heavy-chain gene to the *bcl-1* locus on chromosome 11. However, the identification of the *bcl-1* gene, a putative dominant oncogene, has been elusive. In this work, we have isolated genomic clones covering 120 kb of the *bcl-1* locus. Probes from the region of an *Hpa*II-tiny-fragment island identified a candidate *bcl-1* gene. cDNAs representing the *bcl-1* mRNA were cloned from three cell lines, two with the translocation. The deduced amino acid sequence from these clones showed *bcl-1* to be a member of the cyclin gene family. In addition, our analysis of expression of *bcl-1* in an extensive panel of human cell lines showed it to be widely expressed except in lymphoid or myeloid lineages. This observation may provide a molecular basis for distinct modes of cell cycle control in different mammalian tissues. Activation of the *bcl-1* gene may be oncogenic by directly altering progression through the cell cycle.

Chromosome translocations have been associated with many types of human leukemia and lymphoma. It is accepted that these translocations disrupt proto-oncogenes that are involved in the pathogenesis of these malignancies (4, 14). The analysis of chromosome translocations in leukemia and lymphoma has led to the improved understanding of known oncogenes, such as c-*myc* and c-*abl*, and the discovery of new oncogenes, such as *bcl-2* (2, 6, 19, 40, 42).

The t(11;14)(q13;q32) translocation is an important abnormality associated with B-lymphocytic malignancy (9, 32, 48, 50). Breakpoints at chromosome 14q32 occur in the joining region of the immunoglobulin heavy-chain (IgH) gene (25, 46, 47). Chromosome 11 breakpoints occur in a region called the *bcl-1* (B-cell leukemia/lymphoma 1) locus, covering at least 63 kb of chromosome 11 (15, 25, 35, 46). A high proportion of the documented breakpoints are found in a subregion of the locus called the major translocation cluster (MTC) (25, 46). Although a dominant oncogene in the *bcl-1* locus has been postulated for many years, the identification of the *bcl-1* gene has been elusive.

In one approach to identify the *bcl-1* gene, we have mapped a number of candidate oncogenes from 11q13 by using the radiation hybrid technique (36). This study allowed us to determine whether any known genes mapped close to the *bcl-1* locus. Potential *bcl-1* candidate genes CD5, CD20, c-*sea*, and protein phosphatase 1a were eliminated by this analysis. In part, these data allowed us to conclude that *bcl-1* would be a novel gene.

In this report we present a second approach to identify the *bcl-1* gene. We report the cloning and analysis of 120 kb of genomic DNA from the *bcl-1* locus. We identified a member of the cyclin gene family whose expression is deregulated in two leukemia samples with the t(11;14)(q13;q32) translocation. This gene appears to be the *bcl-1* gene.

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MATERIALS AND METHODS

Cell lines. The four cell lines with the prefix MO were derived from the culture of peripheral blood leukemic cells in the presence of Epstein-Barr virus (27). MO2058 and MO1094 were derived from patients with chronic lymphocytic leukemia (CLL; prolymphocytic variant), and both had the t(11;14)(q13;q32) translocation. The translocations in both of these cell lines have been structurally characterized (27). MO1079 and MO1129 were used as negative controls. These lines were also derived from CLL cells. However, the karyotype of these lines exhibited a trisomy of chromosome 12 without evidence of any chromosome 11 abnormality.

A253 (squamous cell carcinoma), Tera-2 (teratocarcinoma), A431 (cervical carcinoma), and FaDu (esophageal carcinoma) were purchased from the American Type Culture Collection. GM607 (B lymphoblastoid) was obtained from the Human Genetic Mutant Repository. Additional human cell lines included K562 (erythroleukemia), U-937 (monocytic), Reh (B-lineage leukemia), and Jurkat (T lymphocyte).

Southern blots and genomic libraries. Isolation of genomic DNA and Southern blotting were performed as previously described (25). Hybridization to DNAs from heterologous species was performed in 30% formamide. Field inversion gel electrophoresis was performed by using published methods (5, 10). Unless otherwise stated, human genomic DNA was derived from peripheral blood granulocytes. Genomic DNA was also made from mouse liver, rat liver, and pig peripheral blood buffy coat. Cow genomic DNA was provided by Clontech. *Xenopus* DNA was a gift from J. Gautier, and *Saccharomyces cerevisiae* SS13 DNA was a gift from the laboratory of I. Herskowitz.

Genomic libraries were made as previously described (8, 25). Briefly, genomic DNA was partially digested with *Sau*3A and fractionated on a sucrose gradient. Fragments between 15 and 23 kb were ligated into EMBL3, packaged, plated, and screened. Clones with the prefix 514- were derived from a bone marrow sample. This sample contained 80% normal cells and 20% cells from a clonal malignancy without any evidence of chromosome 11 abnormality. The prefix G denotes clones obtained from a normal human male

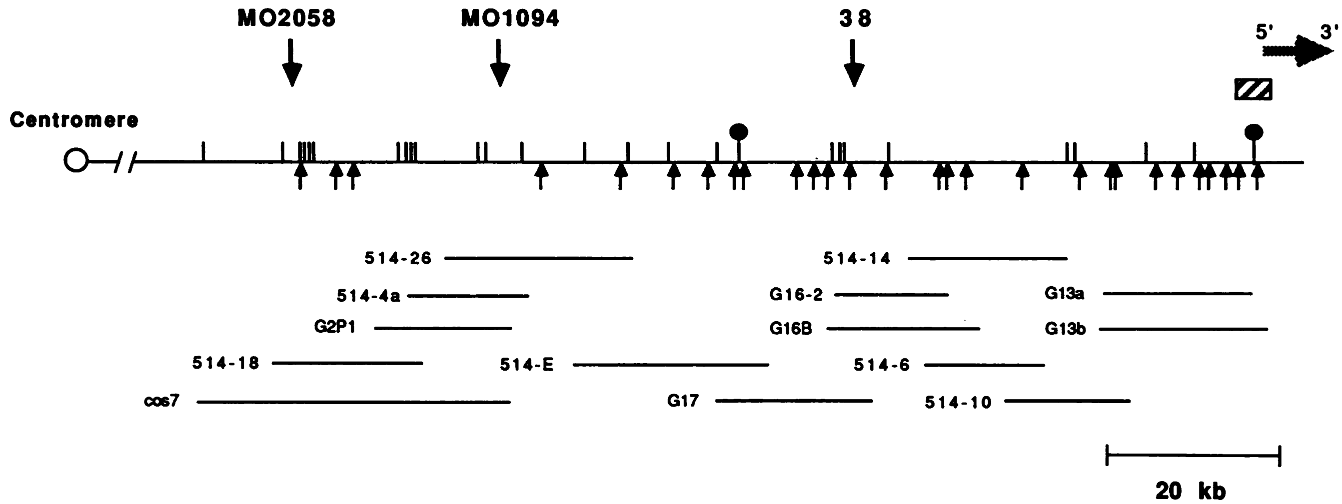

FIG. 1. Diagram representing more than 120 kb of the *bcl-1* locus at human chromosome 11q13. The B4.0 genomic fragment (cross-hatched box) spans an HTF island and identifies the *bcl-1* mRNA. The 5′ end of the *bcl-1* mRNA originates within the B4.0 probe, and transcription proceeds toward the telomere (as shown by the stippled arrow). The translocation breakpoint in the MO2058 cell line occurred within the major translocation cluster, approximately 110 kb from the B4.0 probe. The MO1094 and CLL sample 38 breakpoints are located approximately 85 and 47 kb from the B4.0 probe. All known *Hin*dIII (vertical lines) and *Eco*RI (arrows) restriction sites are indicated, as well as two hypomethylated *Eag*I sites (solid circles). The locations of 13 normal phage clones and one cosmid clone are indicated.

granulocyte library. The cosmid clone cos7 was isolated from a human placental DNA library (Stratagene).

RNA isolation, Northern (RNA) blotting, and RNase protection assay. Poly(A)$^+$ RNA was isolated as previously described (26). RNA was electrophoresed through formaldehyde agarose gels, and Northern blotting was performed by using published methods (26). RNA markers were obtained from Bethesda Research Laboratories. As a control, Northern blots were probed with the *Bgl*I-*Pst*I pHcGAPNR fragment from the glyceraldehyde-3-phosphate dehydrogenase cDNA (45). Single-stranded probes for Northern blotting and protection experiments were generated by using the Bluescript plasmid (Stratagene) with T3 or T7 RNA polymerase (28).

For RNase protection experiments (55), 10 μg of total RNA [or 0.3 μg of poly(A)$^+$ RNA with 10 μg of yeast tRNA] was mixed with probe in 30 μl of hybridization buffer [80% formamide, 40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.4), 400 mM NaCl, 1 mM EDTA]. Samples were heated to 85°C for 5 min and then incubated at 55°C for 12 h. Samples were diluted with 350 μl of RNase digestion mix (10 mM Tris-HCl [pH 7.5], 300 mM NaCl, 5 mM EDTA, 40 μg of RNase A per ml, and 2 μg of RNase T_1 per ml) and incubated at 37°C for 1 h. Proteinase K (to 125 μg/ml) and sodium dodecyl sulfate (SDS; to 0.5%) were added. After 15 min at 37°C, samples were phenol-chloroform extracted, ethanol precipitated, and separated on a 6% denaturing acrylamide gel.

cDNA libraries. Five libraries from three cell lines were made by using lambda gt10 (13). For MO1094, one library was made with oligo(dT) and random hexamer priming using established technology (Clontech) and one library was made by using only oligo(dT) priming of first-strand synthesis and RNase H to assist second-strand synthesis (Stratagene) (11). Two similar libraries were made for MO2058. The A253 library was made with use of oligo(dT) priming by the method of Gubler and Hoffman (11) (Stratagene). cDNA libraries were screened with the B4.0, EH420, and GE370 probes.

Sequencing and computer searches. Sequencing was performed by the chain termination method with M13 and plasmid vectors (17, 54). All sequences represent data obtained from both directions.

Protein data base searches were performed by using FASTA software (version 1.4d, February 1991), obtained as a generous gift from W. Pearson (33). The parameters for searching were as follows: ktup = 2; scoring matrix = PAM250. In a search of the NBRF PIR protein sequence data base, 30,087 sequences were searched against the predicted 295-amino-acid protein from A253. The mean initn score was 25.0 with a standard deviation of 7.21, and the mean initl score was 24.7 with a standard deviation of 6.49. Twelve scores with initn and opt greater than 100 were obtained, and all were cyclins. In the search of a different data base, performed by Glenn Hammonds, 47,645 sequences were searched and the same result was obtained.

Nucleotide sequence accession number. The nucleotide sequence data reported will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under accession number M73554.

RESULTS

Our search for the *bcl-1* gene was based on the hypothesis that the *bcl-1* gene would be associated with the first *Hpa*II-tiny-fragment (HTF) island telomeric of the *bcl-1* locus breakpoints that we had described previously (25, 27). This model was based on previous findings that (i) in translocations involving the IgH locus, the activated oncogene is found upstream of the IgH enhancer, and (ii) genes are frequently associated with HTF islands (21). Therefore, we used the technique of chromosome walking to isolate normal genomic clones extending telomeric on chromosome 11 from the sites of the translocations to the first HTF island (Fig. 1).

Early in the course of this work, we used methylation-sensitive restriction enzymes and field inversion gel electrophoresis to estimate the distance to the first telomeric HTF

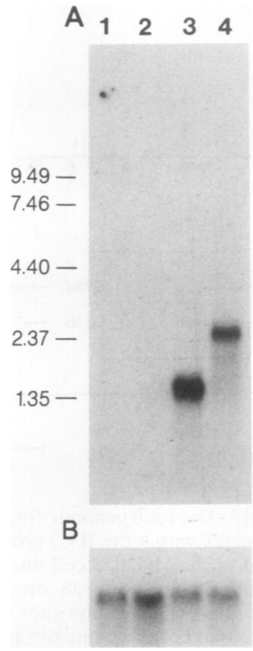

FIG. 2. Expression of the *bcl-1* gene in leukemic cells containing the t(11;14)(q13;q32) translocation. The Northern blot documents that expression of the *bcl-1* gene is associated with the t(11;14)(q13; q32) translocation. (A) Filter containing 5 μg of poly(A)$^+$ RNA in each lane probed with the B4.0 probe. Lanes: 1, MO1129; 2, MO1079; 3, MO1094; 4, MO2058. The bands in lanes 3 and 4 were estimated to be 1.5 and 2.5 kb, respectively, from comparison with RNA markers (sizes in kilobases are indicated). (B) Rehybridization of the filter with a probe from the glyceraldehyde-3-phosphate dehydrogenase gene to document equal loading.

island. We identified an *Eag*I restriction site, 55 kb telomeric of the breakpoint in the MO2058 cell line, that could be digested in genomic DNA from normal human granulocytes and the GM607 lymphoblastoid cell line (Fig. 1). This *Eag*I site was not in an HTF island. Using this restriction site as a landmark, we determined the distance to the next telomeric, hypomethylated *Eag*I site to be 50 to 60 kb (Fig. 1). In addition, we could document no cutting by *Bss*HII, *Sac*II, or *Not*I between these two *Eag*I sites. We concluded that there might be an HTF island approximately 110 kb telomeric of the MTC.

As hypothesized, the first HTF island was located 110 kb telomeric of the MTC and 47 kb telomeric of the breakpoint from CLL sample 38 (the most telomeric of the reported translocation breakpoints) (25). This region is covered by the subcloned B4.0 probe shown in Fig. 1. In a 1.7-kb fragment of this probe, there were two *Sac*II sites, one *Bss*HII site, and a superimposed *Not*I and *Eag*I site. Southern blotting using the B4.0 probe documented that the *Eag*I site was hypomethylated and therefore corresponded to the *Eag*I site predicted in the field inversion gel electrophoresis experiments (data not shown).

To determine whether a gene was associated with this HTF island, a Northern blot using the B4.0 probe was performed. Four cell lines were studied, all derived from patients with CLL (Fig. 2). In cell lines MO2058 and MO1094, both having the t(11;14)(q13;q32) translocation, a distinct transcript was identified. No transcript was evident in the two CLL cell lines without the translocation. In MO2058 the transcript was approximately 2.5 kb long, and in MO1094 it was approximately 1.5 kb long. The presence of a transcript only in the CLL lines containing the translocation strengthened our belief that we had identified the *bcl-1* gene.

To determine the typical size of the *bcl-1* mRNA, we screened a large number of human cell lines by Northern blotting. We anticipated that the most common size of the *bcl-1* mRNA would be either 2.5 or 1.5 kb. Instead, we observed that most human cell lines expressed a 4.4-kb mRNA (accounting for 80 to 90% of the transcripts), with minor bands at 4.2 and 1.5 kb. One representative cell line, A253 (derived from a squamous cell carcinoma), was chosen for detailed study.

To further characterize the *bcl-1* gene, cDNA libraries were made from A253, MO2058, and MO1094. Representative cDNA clones from all three cell lines were sequenced and analyzed. The sequence derived from the A253 clones covers 4,221 bp and contains a consensus polyadenylation signal sequence at the 3′ end (Fig. 3). The sequence of the MO2058 clones spans 2,415 bp and is identical to that of A253 with the following exceptions (positions refer to numbering in Fig. 3): (i) an additional 45 bp (colinear with genomic DNA) are present at the 5′ end; (ii) a C is present at nucleotide 281 instead of G, resulting in a change from cysteine to serine at amino acid 47; (iii) a deletion occurred from nucleotides 1652 to 3503 (1,852 bases in length), (iv) a C is present at nucleotide 3759 instead of T; and (v) an additional C is present at the 3′ end. The sequence of the MO1094 clones covers 1,353 bp and is identical to the A253 sequence except that (i) an additional 6 bp are present at the 5′ end, (ii) nucleotide 68 is T instead of G, (iii) nucleotide 271 is G instead of T (substituting an aspartic acid for tyrosine at amino acid 44), (iv) nucleotide 864 is A instead of G, and (v) a consensus polyadenylation signal starting at nucleotide 1333 is generated by a 3-bp deletion (AATAATCAACTC to AATAAACTC).

The *bcl-1* mRNAs from A253, MO2058, and MO1094 are compared schematically in Fig. 4. All three have the *bcl-1* open reading frame (ORF) in common. This ORF starts at a methionine codon in a favored context for translation initiation (16). The ORF extends for 885 nucleotides and predicts a protein of 295 amino acids. All three forms of *bcl-1* are different in the 3′ untranslated region. A253 represents the typical transcript with a long 3′ untranslated region. MO2058 has undergone an internal loss of 1,852 bp of the 3′ untranslated region. Junctional sequences do not correspond to consensus splice signals (39). In addition, a Southern blot of MO2058 demonstrates that this loss has resulted from a genomic deletion (data not shown). MO1094 is truncated in the proximal 3′ untranslated region by the introduction of a new polyadenylation signal sequence.

The relationship of the cDNA clones to the genomic map was determined. A selected region of approximately 500 bp from B4.0 was sequenced. It contained the 5′ end of the cDNA clones from all three cell lines. From these data we determined that the 5′ end of *bcl-1* is centromeric and that transcription proceeds toward the telomere (Fig. 1). This result was confirmed by using Northern blotting with single-stranded probes from the B4.0 region (data not shown).

RNase protection assays were performed to determine the exact *bcl-1* transcription start site within B4.0. An antisense genomic probe extending 5′ from the *Nco*I site (location of translation initiation) was hybridized to RNA from several cell lines and subsequently digested with RNases A and T_1. In MO2058, MO1094, A253, and A431, a protected fragment of approximately 160 nucleotides was detected (Fig. 5).

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GTAGCAGCGAGCAGCAGAGTCCGCACGCTCCGGCGAGGGGCAGAAGAGCGCGAGGGAGCGCGGGGCAGCAGAAGCGAGAGCCGAGCGCGG   90
ACCCAGCCAGGACCCACAGCCCTCCCCAGCTGCCCAGGAAGAGCCCCAGCCATGGAACACCAGCTCCTGTGCTGCGAAGTGGAAACCATC  180
                                                   M  E  H  Q  L  L  C  C  E  V  E  T  I

CGCCGCGCGTACCCCGATGCCAACCTCCTCAACGACCGGGTGCTGCGGGCCATGCTGAAGGCGGAGGAGACCTGCGCGCCCTCGGTGTCC  270
 R  R  A  Y  P  D  A  N  L  L  N  D  R  V  L  R  A  M  L  K  A  E  E  T  C  A  P  S  V  S

TACTTCAAATGTGTGCAGAAGGAGGTCCTGCCGTCCATGCGGAAGATCGTCGCCACCTGGATGCTGGAGGTCTGCGAGGAACAGAAGTGC  360
 Y  F  K  C  V  Q  K  E  V  L  P  S  M  R  K  I  V  A  T  W  M  L  E  V  C  E  E  Q  K  C

GAGGAGGAGGTCTTCCCGCTGGCCATGAACTACCTGGACCGCTTCCTGTCGCTGGAGCCCGTGAAAAAGAGCCGCCTGCAGCTGCTGGGG  450
 E  E  E  V  F  P  L  A  M  N  Y  L  D  R  F  L  S  L  E  P  V  K  K  S  R  L  Q  L  L  G

GCCACTTGCATGTTCGTGGCCTCTAAGATGAAGGAGACCATCCCCCTGACGGCCGAGAAGCTGTGCATCTACACCGACAACTCCATCCGG  540
 A  T  C  M  F  V  A  S  K  M  K  E  T  I  P  L  T  A  E  K  L  C  I  Y  T  D  N  S  I  R

CCCGAGGAGCTGCTGCAAATGGAGCTGCTCCTGGTGAACAAGCTCAAGTGGAACCTGGCCGCAATGACCCCGCACGATTTCATTGAACAC  630
 P  E  E  L  L  Q  M  E  L  L  L  V  N  K  L  K  W  N  L  A  A  M  T  P  H  D  F  I  E  H

TTCCTCTCCAAAATGCCAGAGGCGGAGGAGAACAAACAGATCATCCGCAAACACGCGCAGACCTTCGTTGCCCTCTGTGCCACAGATGTG  720
 F  L  S  K  M  P  E  A  E  E  N  K  Q  I  I  R  K  H  A  Q  T  F  V  A  L  C  A  T  D  V

AAGTTCATTTCCAATCCGCCCTCCATGGTGGCAGCGGGGAGCGTGGTGGCCGCAGTGCAAGGCCTGAACCTGAGGAGCCCCAACAACTTC  810
 K  F  I  S  N  P  P  S  M  V  A  A  G  S  V  V  A  A  V  Q  G  L  N  L  R  S  P  N  N  F

CTGTCCTACTACCGCCTCACACGCTTCCTCTCCAGAGTGATCAAGTGTGACCCGGACTGCCTCCGGGCCTGCCAGGAGCAGATCGAAGCC  900
 L  S  Y  Y  R  L  T  R  F  L  S  R  V  I  K  C  D  P  D  C  L  R  A  C  Q  E  Q  I  E  A

CTGCTGGAGTCAAGCCTGCGCCAGGCCCAGCAGAACATGGACCCCAAGGCCGCCGAGGAGGAGGAAGAGGAGGAGGAGGAGGTGGACCTG  990
 L  L  E  S  S  L  R  Q  A  Q  Q  N  M  D  P  K  A  A  E  E  E  E  E  E  E  E  E  V  D  L

GCTTGCACACCCACCGACGTGCGGGACGTGGACATCTGAGGGCGCCAGGCAGGCGGGCGCCACCGCCACCCGCAGCGAGGGCGGAGCCGG 1080
 A  C  T  P  T  D  V  R  D  V  D  I  *

CCCCAGGTGCTCCACTGACAGTCCCTCCTCTCCGGAGCATTTTGATACCAGAAGGGAAAGCTTCATTCTCCTTGTTGTTGGTTGTTTTTT 1170
CCTTTGCTCTTTCCCCCTTCCATCTCTGACTTAAGCAAAAGAAAAAGATTACCCAAAAACTGTCTTTAAAAGAGAGAGAGAGAAAAAAAA 1260
AATAGTATTTGCATAACCCTGAGCGGTGGGGGAGGAGGGTTGTGCTACAGATGATAGAGGATTTTATACCCCAATAATCAACTCGTTTTT 1350
ATATTAATGTACTTGTTTCTCTGTTGTAAGAATAGGCATTAACACAAAGGAGGCGTCTCGGGAGAGGATTAGGTTCCATCCTTTACGTGT 1440
TTAAAAAAAAGCATAAAAACATTTTAAAAACATAGAAAAATTCAGCAAACCATTTTTAAAGTAGAAGAGGGTTTTAGGTAGAAAAACATA 1530
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CAGCCAGCGTAGCAGGGTCGGGAAAGGCCACCTGTCCCACTCCTACGATACGCTACTATAAAGAGAAGACGAAATAGTGACATAATATAT 1890
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TATAGTTAAGGCTAAAAAGTATATTTATTGCAGAGGATGTTCATAAGGCCAGTATGATTTATAAATGCAATCTCCCCTTGATTTAAACAC 2520
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TAGTGACAAAATAGACAATTTGCACATCTTGGCTATGTAATTCTTGTAATTTTTATTTAGGAAGTGTTGAAGGGAGGTGGCAAGAGTGTG 2880
GAGGCTGACGTGTGAGGGAGGACAGGCGGGAGGAGGTGTGAGGAGGAGGCTCCCGAGGGGAAGGGGCGGTGCCCACACCGGGGACAGGCC 2970
GCAGCTCCATTTTCTTATTGCGCTGCTACCGTTGACTTCCAGGCACGGTTTGGAAATATTCACATCGCTTCTGTGTATCTCTTTCACATT 3060
GTTTGCTGCTATTGGAGGATCAGTTTTTTGTTTTACAATGTCATATACTGCCATGTACTAGTTTTAGTTTTCTCTTAGAACATTGTATTA 3150
CAGATGCCTTTTTTGTAGTTTTTTTTTTTTTTTTATGTGATCAATTTTGACTTAATGTGATTACTGCTCTATTCCAAAAAGGTTGCTGTT 3240
TCACAATACCTCATGCTTCACTTAGCCATGGTGGACCCAGCGGGCAGGTTCTGCCTGCTTTGGCGGGCAGACACGCGGGCGCGATCCCAC 3330
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CATAATGCTAATTTAAAGAGACTCCAAATCTCAATGAAGCCAGCTCACAGTGCTGTGTGCCCCGGTCATCTAGCAAGCTGCCGAACCAAA 3780
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ACGCTTTGTCTGTCGTGATGGGGCAAGGGCACAAGTCCTGGATGTTGTGTGTATCGAGAGGCCAAAGGCTGGTGGCAAGTGCACGGGGCA 4050
CAGCGGAGTCTGTCCTGTGACGCGCAAGTCTGAGGGTCTGGGCGGCGGGCGGCTGGGTCTGTGCATTTCTGGTTGCACCGCGGCGCTTCC 4140
CAGCACCAACATGTAACCGGCATGTTTCCAGCAGAAGACAAAAAGACAAACATGAAAGTCTAGAAATAAAACTGGTAAAAC          4221
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FIG. 3. *bcl-1* sequence derived from the A253 cDNA clones (4,221 bp). The 295-amino-acid coding region is shown. The polyadenylation signal sequence is underlined.

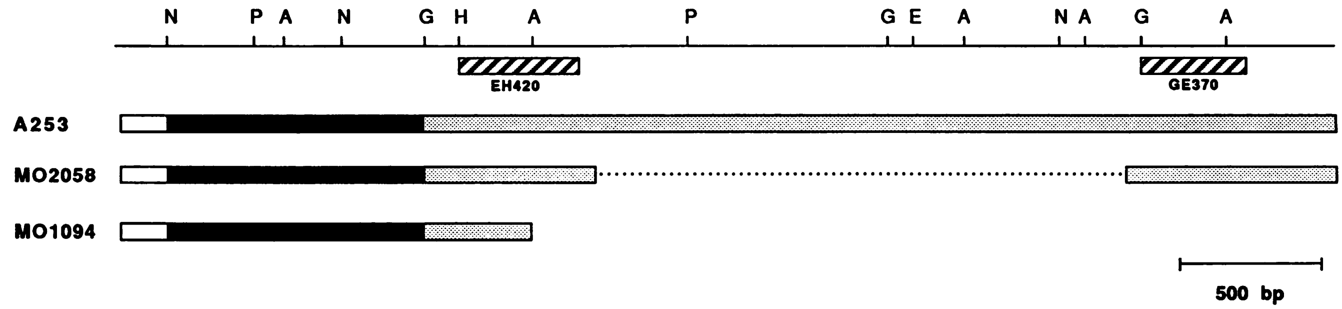

FIG. 4. Schematic comparing the three forms of the *bcl-1* cDNA. The structure exhibited by A253 is the typical form of the *bcl-1* mRNA in most human cell lines. The *bcl-1* mRNA in MO2058 results from the loss of 1,852 bp in the 3′ untranslated region (dotted line). The *bcl-1* mRNA in MO1094 terminates as the result of a new polyadenylation signal sequence in proximal 3′ untranslated sequences. The open box represents the 5′ untranslated sequences, the solid box represents the protein-coding region, and the stippled box represents the 3′ untranslated sequences. Cross-hatched boxes represent probes derived from MO2058 cDNA clones. Restriction sites: A, *Ava*I; E, *Eco*RI; G, *Bgl*I; H, *Hin*dIII; N, *Nco*I; P, *Pst*I.

When considered with the fact that no isolated cDNA clone (of more than 20 analyzed) showed any evidence for the presence of an upstream exon, this experiment defines the primary start site for transcription. From the genomic map, it is clear that all t(11;14)(q13;32) translocations described so far occur outside the transcribed region of the *bcl-1* gene.

The *bcl-1* gene was found to be highly conserved in evolution. Relatively stringent Southern blotting was performed, and signals from mouse, rat, cow, and pig were easily detected (Fig. 6). In work with the human *Pim-1* gene under similar conditions, hybridization to mouse *Pim-1* was not detected despite 89% overall nucleotide homology (24) (unpublished data). Therefore, we would anticipate that the human *bcl-1* nucleotide sequence is very similar to the mouse, rat, cow, and pig sequences. *Drosophila* DNA had a weak signal in our experiments, while *Xenopus* and *S. cerevisiae* DNAs had no detectable signal.

The deduced protein sequence from A253 was used to search the available data bases. The *bcl-1* protein was found to have significant homology to several cyclins (7, 31, 43). In our analysis, the highest similarity was to A-type cyclins from human, African clawed frog, Atlantic surf clam, and

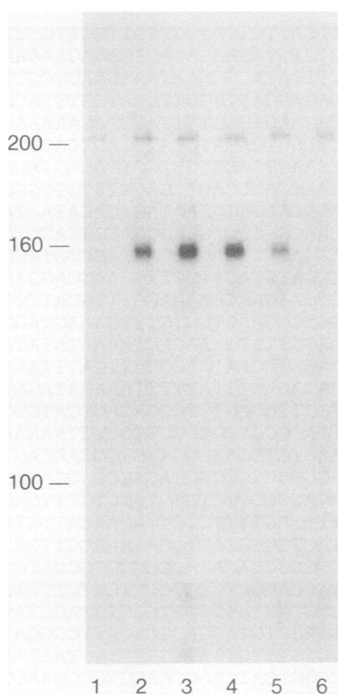

FIG. 5. *bcl-1* transcription initiation site. In an effort to identify the major site of transcription initiation, an RNase protection assay was performed. RNA samples were incubated with a 700-nucleotide antisense genomic probe extending from the *Nco*I site (location of translation initiation). In MO2058, MO1094, A253, and A431 (lanes 2 to 5, respectively), a major protected fragment of 160 nucleotides was detected. In MO2058 and MO1094, minor protected fragments could be seen migrating at about 172 and 195 bases. In K562 (lane 6), no protected fragment could be detected with this assay. Yeast tRNA (lane 1) served as a negative control.

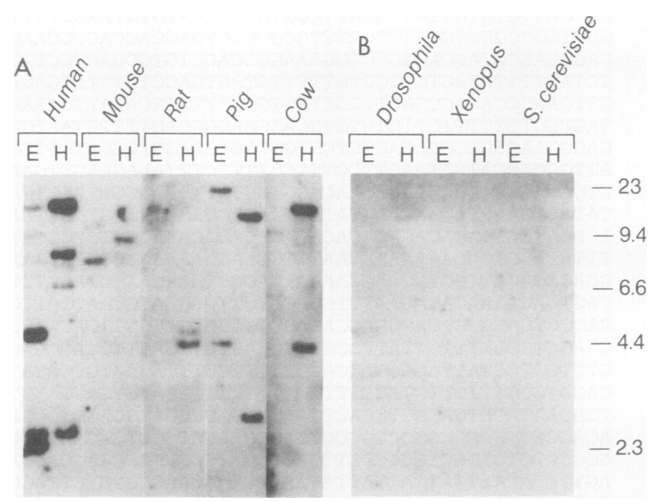

FIG. 6. Assessment of evolutionary conservation. Two Southern blot analyses were performed to assess the amount of nucleotide conservation during evolution. Ten micrograms of genomic DNA was loaded in each lane. Both blots were hybridized with an MO2058 cDNA clone. Blot A was washed to a final stringency of 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 65°C; blot B was washed to 1× SSC–0.1% SDS at 55°C. The results document that human, mouse, rat, pig, and cow cells have a highly conserved *bcl-1* gene (estimated at greater than 90% nucleotide homology). In *Drosophila* DNA there appears to be a low-level signal, while in *Xenopus* and *S. cerevisiae* DNAs there is no detectable signal. Marker sizes in kilobases are indicated.

```
          ** *   *  ** ** *   *   *   * *****   *    *** *      *
1  (55)   MRKIVATWMLEVCEEQKCEEEVFPLAMNYLDRFLSLEPVKKSRLQLLGATCMFVA
2  (209)  --A-LVD-LV--G--Y-LQN-TLH--V--I-----SMS-LRGK---V-TAA-LL-
3  (195)  --T-LVD-LV--G--Y-LHT-TLY-----------CMS-LRGK---V-TAAILL-
4  (194)  --C-LVD-LV--S--D-LHR-TLF-GV--I-----KIS-LRGK---V--AS--L-
5  (234)  --S-LID-LV--S--Y-LDT-TLY-SVF-------QMA-VR-K---V-TAA-YI-

           *  *  *         **        * **      *       *   *
1         SKMKETIPLTAEKLCIYTDNSIRPEELLQMELLLVNKLKWNLAAMTPHDFIEHFL (130)
2         --FE-IY-PEVAEFVYI--DTYTKKQV-R--H-VLKV-TFD---P-VNQ-LTQYF (113)
3         --YE-IY-PDVDEFVYI--DTYSKKQ--R--HV-LKV-AFD-TVP-VNQ-LLQY- (113)
4         A-YE-IY-PDVKEFAYI--DTYTSQQV-R--H-ILKV-TFDV-VP-TNW-C-D-- (118)
5         A-YE-IY-PEVGEFVFL--D-YTKAQV-R--QVILKI-SFD-CTP-AYV--NTYA (147)
```

FIG. 7. Comparison of the *bcl-1* protein with four A-type cyclins. The *bcl-1* protein (row 1) is aligned with cyclin A from human (row 2), African clawed frog (*Xenopus laevis*; row 3), Atlantic surf clam (*Spisula solidissima*; row 4), and fruit fly (*Drosophila melanogaster*; row 5) cells (20, 29, 34, 43, 49, 51). The compared region spans 110 amino acids (aligned without gapping) and includes the cyclin box. A dash indicates an identical amino acid; an asterisk over a column indicates an amino acid conserved in all five sequences. The numbers in parentheses indicate the number of amino acids for each sequence before and after the region shown.

fruit fly cells. Significant similarities to B-type cyclins were also detected. A comparison of the *bcl-1* protein sequence with the four most similar protein sequences is shown in Fig. 7. These data support the inclusion of *bcl-1* in the cyclin family.

We have used Northern blotting of poly(A)$^+$ RNA to survey an extensive panel of human cell lines for *bcl-1* expression (Fig. 8). Although *bcl-1* is expressed by cell lines from many different tissues, no cell line representing a lineage derived from the bone marrow stem cell (lymphoid or myeloid) exhibited detectable expression. Furthermore, RNase protection assays of K562 exhibited no *bcl-1* expression (Fig. 5). The only exceptions to the pattern were cell lines with the t(11;14)(q13;q32) translocation. All cell lines studied are rapidly growing in culture, so we do not believe that this difference resulted from the number of cells in cycle or the cell cycle kinetics in these cultures. Our data indicate that *bcl-1* is expressed at different levels in cell lines derived from different mammalian tissues.

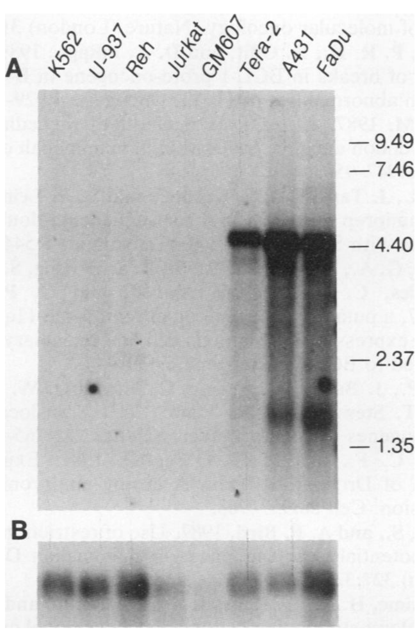

FIG. 8. Selective expression of *bcl-1* in human cell lines. A representative Northern blot containing 5 μg of poly(A)$^+$ mRNA in each lane is shown after probing for *bcl-1* expression with an MO2058 cDNA clone. The major transcript at 4.4 kb is detected in three of these cell lines (Tera-2, A431, and FaDu). Importantly, *bcl-1* mRNA is not detected in any myeloid or lymphoid cell line (K562, U-937, Reh, Jurkat, and GM607).

DISCUSSION

Abnormalities of the *bcl-1* locus are important in several subtypes of B-lymphocytic leukemia and lymphoma (23, 37, 52). Exploration of the pathophysiology of these diseases has awaited the identification of the *bcl-1* gene. In this work, we explored the hypothesis that the *bcl-1* gene may be associated with the first HTF island telomeric of the known *bcl-1* breakpoints. As a result, we identified an HTF island 110 kb from the major translocation cluster and 47 kb telomeric of the breakpoint in CLL38. Probes from the region of this HTF island have identified a gene. Three pieces of data argue that this is the *bcl-1* gene. First, it is the first known gene telomeric of the translocation breakpoints. Second, it is activated in cell lines with the t(11;14)(q13;q32) translocation. Third, as a member of the cyclin family, its perturbation might be expected to lead to altered cell cycle progression.

The *bcl-1* gene has recently been identified by other groups using different strategies. In parathyroid adenomas, it has been shown that this gene (called PRAD1) is occasionally activated by a pericentric inversion of chromosome 11 (30, 38). Xiong and coworkers found the *bcl-1* gene (called the cyclin D1 gene) in a screen for human genes that complement yeast cells deficient in G_1 cyclin activity (53). Matsushime and coworkers found the apparent murine homolog of *bcl-1* (called *Cyl-1*) by screening a mouse macrophage cell line for genes that might regulate G_1 progression in the presence of colony-stimulating factor 1 (22).

The accumulated evidence suggests that the *bcl-1* protein structurally belongs to the cyclin family and functions as a cyclin. In this work, analysis of the deduced *bcl-1* amino acid sequence showed the protein to be a member of the cyclin family (7, 31, 43). The highest similarity was found to A-type cyclins, although significant similarities to B-type cyclins were also detected. Interestingly, the *bcl-1* protein differs from other cyclins in two obvious ways. First, *bcl-1* is the smallest known member of the cyclin family, being significantly shorter at the amino terminus. Second, *bcl-1* has nine consecutive glutamic acid residues close to the carboxy terminus. Further study will reveal the functional correlates of these two features. As membership in the cyclin family might predict, it has been shown by others that *bcl-1* mRNA and protein are expressed in relationship to the cell cycle, and the *bcl-1* protein binds $p34^{cdc2}$ or closely related molecules (22, 30).

The t(11;14)(q13;q32) translocation appears to activate *bcl-1* by increasing levels of *bcl-1* mRNA. All known translocation breakpoints fall outside the *bcl-1* transcriptional unit. Therefore, there is no evidence for a fusion transcript or a fusion protein, as described for some other translocations. The elevated *bcl-1* mRNA levels might result from an interaction between the IgH enhancer and the *bcl-1* promoter, as described in some translocations involving the c-*myc* oncogene (12). This hypothesis would require activity of the IgH enhancer at distances up to 110 kb of genomic DNA, as suggested for some translocations that activate c-*myc* (41). However, it is also possible that the translocation eliminates a distant negative control element, leading to *bcl-1* activation.

The different sizes of *bcl-1* mRNA in the three cell lines resulted from different 3′ untranslated structures. In most cell lines that we have studied (for example, A253), the *bcl-1* gene is expressed primarily as a 4.4-kb transcript. In contrast, the major 4.4-kb transcript was absent in both leukemia cell lines with the translocation. In the MO2058 cell line we detected a 2.5-kb transcript, and in the MO1094 cell line we found overexpression of a 1.5-kb transcript. In the three cell lines studied, the transcription start sites and protein-coding regions were the same (except for an occasional point mutation). The transcript in MO2058 resulted from the deletion of 1,852 nucleotides in the 3′ untranslated region, and the transcript in MO1094 was truncated by the introduction of a new polyadenylation signal sequence. The data suggest that the loss of sequences in the 3′ untranslated region of *bcl-1* represents an additional aspect of activation, possibly by altering mRNA stability.

We present evidence that *bcl-1* may be selectively expressed in different tissues. Most human cell lines in our panel had easily detectable levels of *bcl-1* mRNA. In contrast, *bcl-1* mRNA was undetectable in cell lines representing lineages derived from bone marrow stem cells (whether lymphoid or myeloid). Selective cyclin expression may represent one way in which different mammalian tissues adapt to specialized requirements for cell division.

Although our major interest is the role of *bcl-1* in B-lymphocytic leukemia and lymphoma, the *bcl-1* locus has been implicated in several other types of cancer. Amplifications of *bcl-1* are detected in approximately 20% of breast cancer and squamous cell cancers (1, 3, 44). We and others have data to indicate that *bcl-1* is expressed in human tumor cell lines representing these tumor types (18). It will be important to determine whether the *bcl-1* gene has a pathogenic role in a wide spectrum of cancers.

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